Eukaryotic DNA mismatch repair

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Eukaryotic mismatch repair (MMR) has been shown to require two different heterodimeric complexes of MutS-related proteins: MSH2–MSH3 and MSH2–MSH6. These two complexes have different mispair recognition properties and different abilities to support MMR. Alternative models have been proposed for how these MSH complexes function in MMR. Two different heterodimeric complexes of MutL-related proteins, MLH1–PMS1 (human PMS2) and MLH1–MLH3 (human PMS1) also function in MMR and appear to interact with other MMR proteins including the MSH complexes and replication factors. A number of other proteins have been implicated in MMR, including DNA polymerase δ, RPA (replication protein A), PCNA (proliferating cell nuclear antigen), RFC (replication factor C), Exonuclease 1, FEN1 (RAD27) and the DNA polymerase δ and ε associated exonucleases. MMR proteins have also been shown to function in other types of repair and recombination that appear distinct from MMR. MMR proteins function in these processes in conjunction with components of nucleotide excision repair (NER) and, possibly, recombination.

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Abbreviations

HNPCC	hereditary nonpolyposis colorectal carcinoma
MLH	MutL homologue
MMR	mismatch repair
MSH	MutS homologue
NER	nucleotide excision repair
PCNA	proliferating cell nuclear antigen

Introduction

Since the discovery that hereditary nonpolyposis colorectal carcinoma (HNPCC) is caused by inherited mutations in some of the genes encoding components of a DNA mismatch repair (MMR) pathway [1], there has been extensive progress in identifying and understanding the proteins that function in MMR in eukaryotes. It is the purpose of this article to review some of the recent advances in our understanding of MMR made using *Saccharomyces cerevisiae*, human and mouse systems. As this work has been so highly influenced by previous studies of MMR in bacteria, the reader is referred to reviews covering MMR there [2,3] as well as other general reviews on this process [4–8]. Similarly, although some aspects of the human genetics of MMR will be discussed here, the reader is referred to other reviews for a more comprehensive treatment of this subject [1,4]. (*S. cerevisiae* gene and protein names are used in this article with human names, when they differ, indicated in parenthses.)

MutS homologue proteins involved in mismatch repair

Genetic and protein–protein interaction experiments led to the proposal of a model for eukaryotic MMR in which mispaired bases in DNA are recognized by heterodimeric complexes of MutS-related proteins, the MSH2–MSH6 (MutSα) and MSH2–MSH3 (MutSβ) complexes [4,9] (Figure 1). Studies of the mutator phenotypes caused by mutations in the *Saccharomyces cerevisiae* genes encoding these proteins suggested that the MSH2–MSH6 complex was responsible for the repair of base:base mispairs [9], that the MSH2–MSH6 and MSH2–MSH3 complexes were redundant for the repair of single base insertion/deletion mispairs [9,10], and that the MSH2–MSH3 complex was primarily responsible for the repair of larger insertion/deletion mispairs [4,11•]. This general view has been supported by most studies of MMR and mutator phenotypes using human tumor cells [4,12,13], and mouse cells containing mutations in MMR genes [14•], as well as by studies defining the regions of interaction between MSH2 and both MSH3 and MSH6 [15].

A recent study [16] has suggested that the MSH complexes, in particular the MSH2–MSH6 complex, may have a broader ability to function in the repair of mispaired bases than was anticipated from genetic studies. In this study, the ability of purified human MSH2–MSH6 and human MSH2–MSH3 to complement extracts of LoVo cells that were effectively depleted for MSH2, MSH3 and MSH6 were tested. MSH2–MSH6 was able to support the repair of base:base mispairs and insertion/deletion mispairs having up to 12 unpaired bases whereas MSH2–MSH3 was only able to support the repair of insertion/deletion mispairs having two or more unpaired bases and was not able to support the repair of single base insertion/deletion mispairs as indicated by other studies. Several possible explanations exist for the differences between this and other studies. First, in this study only a single one base insertion/deletion mispair substrate was studied. Given the variable ability of the MSH complexes to recognize different DNA mispair substrates (see below), it is possible that the substrate tested simply represented an example of a substrate that was not recognized by MSH2–MSH3. Second, although genetic studies of *msh6* mutants have suggested that MSH2–MSH6 does not recognize larger insertion/deletion mispairs [10,11•], these genetic studies measured the effect of loss of MSH6 function in the presence of MSH2–MSH3, which efficiently recognizes the

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larger insertion/deletion mispairs. In contrast, the biochemical studies described above measured the ability of MSH2–MSH6 to function under optimal conditions in the absence of MSH2–MSH3 where it would be possible to observe the activity of MSH2–MSH6 on larger insertion/deletion mispairs.

An interesting question relating to the function of the MutS-related proteins is how large an insertion/deletion mispair *MSH2*-dependent MMR will recognize. It has been observed that *msh2*, *msh3* and *msh6* mutations do not cause microsatellite instability of 20 base repeating sequences [11•]. This has suggested that *MSH2*-, *MSH3*-, and *MSH6*-dependent MMR is unlikely to recognize insertion/deletion mispairs having 20 unpaired bases in *S. cerevisiae*. It must be recognized, however, that mutator phenotypes are caused by the combined action of the processes that create mutagenic errors, and the processes that either repair or do not repair the errors. Thus, it is possible that the reason that mutations in *MSH2*, *MSH3* or *MSH6* have no effect on the stability of microsatellites containing 20 base repeating sequences is either that the errors that occur during replication of such sequences do not result in the formation of mispaired bases (e.g. stalled replication forks being converted to double-strand breaks [17•]), or that large insertion/deletion mispairs that might be produced by replication slippage on such repeating sequences are repaired more efficiently by some other process (e.g. recombination). In this regard, evidence has been presented for *MSH2*-dependent repair of 26 and 30 base insertion/deletion mispairs during *S. cerevisiae* meiosis [18,19] and for *MSH2*-, *MSH3*- and *MSH6*-dependent repair of a 38 base insertion/deletion mispair in mitotic *S. cerevisiae* cells [20]. In contrast, the above-mentioned biochemical studies detected highly efficient MSH2–MSH6 and MSH2–MSH3 independent repair of large insertion/deletion mispairs that might have obscured the less-efficient MSH2–MSH3- and MSH2–MSH6-dependent repair of larger insertion/deletion mispairs.

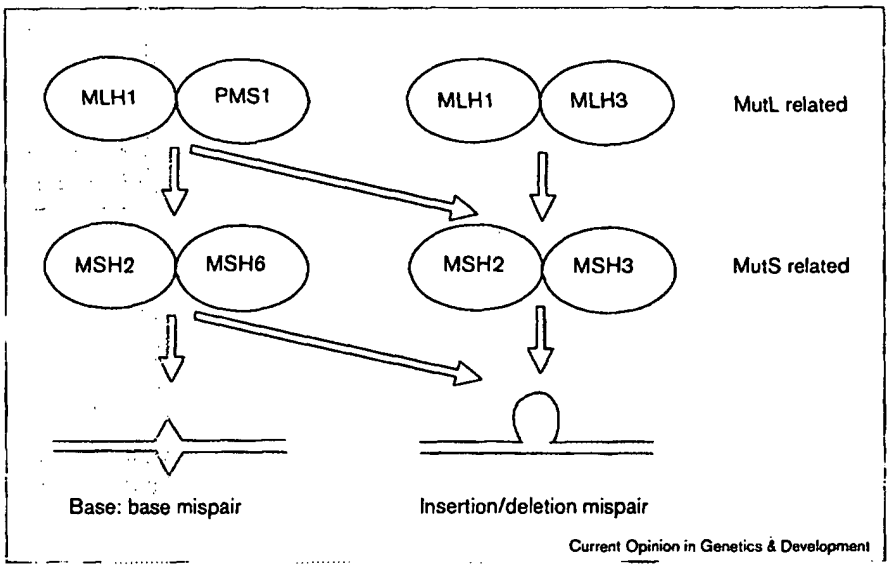
Several studies have investigated the mispair-recognition properties of the MSH2–MSH6 and MSH2–MSH3 complexes. In general, the observed binding specificities for each of the MSH complexes fit with the proposed specificities based on the results of the genetic experiments described above — that is, MSH2–MSH6 binds to G:T mispairs and to +1 insertion deletion/loop mispairs, and MSH2–MSH3 binds to +1 and larger insertion deletion/loop mispairs [21–25]. It should be emphasized, however, that these studies surveyed the recognition of a limited number of DNA mispairs, and only included insertion/deletion loops of 10 bases or less for which *in vivo* repair properties are not known. MSH2–MSH6 binding to palindromic insertion/deletion loop mispairs containing 12 and 14 base loops known to be poorly repaired *in vivo* has also been demonstrated [25]. In a more extensive study of mispair binding of *S. cerevisiae* MSH2–MSH6, we have found that it is capable of recognizing a number of larger (>8 bases) insertion/deletion loop mispairs with an affinity near equal to that of a +1 insertion/deletion loop mispair (GT Marsischky, RD Kolodner, unpublished data). We also observed that MSH2–MSH6 recognizes base:base mispairs with varying efficiency (a G:T mispair was best recognized, whereas a C:C mispair was recognized no better than homoduplex DNA) and that the efficiency of recognition of some base:base mispairs, such as A:C and A:G, could be modified considerably by changes in sequence context. Other studies (e.g. [26]) have also suggested effects of sequence context on mispair recognition.

One of the most highly conserved regions of the MSH2, MSH3 and MSH6 amino acid sequences is the region containing a Walker Type A nucleotide binding fold [27]. Consistent with this, MSH2–MSH6 has been shown to be an ATPase that is stimulated by DNA and in some cases by DNA containing a mispaired base [25,28•,29]. Mutations causing amino acid substitutions in the ATPase consensus sequences in both the MSH2 and MSH6 subunits of the human MSH2–MSH6 complex results in a reduced ATPase activity and a reduced capacity to promote MMR *in vitro* [30]. Such a mutation in the *S. cerevisiae* MSH2 ATPase consensus sequence results in a MSH2-MSH6 complex that retains its ability to bind to mispaired bases in DNA but is nonfunctional for MMR and causes a dominant mutator phenotype when expressed in *S. cerevisiae* [29].

Addition of ATP to MSH2–MSH6 complex mispair binding reactions results in reduced recovery of MSH2–MSH6/mispair complexes although it is not known if ATP reduces complex formation or increases dissociation of the complexes [21,29]. There are two different proposed views of the role of the interaction between MSH2–MSH6 and nucleotides like ATP in MMR. Modrich and co-workers have suggested that ATP hydrolysis is required for translocation/looping of the DNA by MSH2–MSH6 [31•] to coordinate mispair recognition and excision as suggested by the looping model for the mechanism for MutS-dependent MMR in *E. coli* [32•]. Fishel and co-workers have proposed a novel alternative hypothesis in which MSH2–MSH6 exists in an ADP-bound form because when MSH2–MSH6 binds ATP in the absence of a mispaired base, the ATP is hydrolyzed to ADP but the ADP cannot dissociate [28•,33]. This MSH2–MSH6(ADP) form is competent for mispair recognition upon which the mispaired base serves as a cofactor that allows release of ADP and exchange for ATP. ATP binding to MSH2–MSH6, independent of ATP hydrolysis, converts MSH2–MSH6 to a form that disassociates from the mispair, slides along the DNA (Gradia *et al.*, personal communication) and signals or interacts with other components of the MMR machinery. The identity of the components of MMR that are signaled to and what the result of the signaling is have not yet been directly demonstrated.

One striking feature of the data of the mispair binding properties of the MSH2–MSH6 and MSH2–MSH3 complexes is the contrast between the relatively low-affinity differences

Figure 1



Interactions between complexes of MutS-related proteins and complexes of MutL-related proteins during MMR. The studies documenting the indicated interactions between proteins and between protein complexes and mispaired bases are discussed in the text. The gene/protein names used are for *S. cerevisiae*. The closest human homologue of *S. cerevisiae* PMS1 is human PMS2 and the closest human homologue of *S. cerevisiae* MLH3 is human PMS1.

for binding to normal base pairs and mispairs *in vitro* (only 10–20-fold maximal affinity differences are seen between homoduplex and mispaired duplex DNA) ([25,28•,30]; GT Marsischky, RD Kolodner, unpublished data) and the more striking requirement for distinguishing between normal base pairs and mispairs *in vivo*, where the mutation rates in MMR-defective *S. cerevisiae* cells suggest the efficiency of mismatch detection and repair approaches one mispair per cell per generation. There are several possible explanations which might account for the increased MMR specificity observed *in vivo*. First, the MSH complexes might interact directly with other proteins implicated in MMR and these complexes may have increased affinity for mispairs. Second, by analogy to the recognition of transcriptional promoters by proteins and signal transduction, interaction of the MSH complexes with a mispair may cause a conformational change that facilitates protein–protein contacts required to build a higher-order protein–protein structure at the site of the mispair that is required for MMR. Such complexes need not be assembled at the mispair once initial binding has occurred, nor need they remain at the mispair once assembly has occurred. Indeed, the interaction of MutL with MutS, and the MLH1–PMS1 complex with MSH-mispair complexes could possibly represent such an example. Third, and finally, the MSH2–MSH6 signaling hypothesis discussed above could provide a mechanism for increased *in vivo* mispair recognition specificity.

Other MutS homologue proteins

Analysis of the *S. cerevisiae* genome indicated the presence of six genes, *MSH1–6*, encoding proteins related to MutS. *MSH2*, *3* and *6* have been discussed above. To date, no higher eukaryotic homologue has been reported for MSH1, which has been shown in *S. cerevisiae* to function in mitochondrial genome stability [34,35] but higher eukaryotic homologues of MSH4 and MSH5 have been reported [36–38]. In *S. cerevisiae*, MSH4 and MSH5 do not function in MMR but rather are required for crossing over during meiotic recombination where they are thought to function with MLH1 [39–41]. Consistent with this, human MSH4 and MSH5 are most highly expressed in meiotic tissues [36–38] and mutations in mouse *MSH5* cause meiotic defects consistent with MSH5 being required for meiotic recombination [42].

MutL homologue proteins involved in mismatch repair

Genetic and protein–protein interaction experiments led to the proposal of a model for eukaryotic MMR in which a heterodimeric complex of MutL-related proteins, the MLH1–PMS1 (PMS2 in humans) complex interacts with MSH2-containing complexes bound to mispaired bases [43,44] (See Figure 1). Recently, regions of interaction between PMS1 and MLH1 have been mapped [45] and several studies have demonstrated interactions between MutS and MutL homologues. An interaction of *S. cerevisiae* MLH1–PMS1 with MSH2–MSH3 was demonstrated because MLH1–PMS1 can bind a MSH2–MSH3-mispair complex and convert it to a higher molecular weight form [46]. The interaction between human MLH1–PMS2 (PMS1 in *S. cerevisiae*) and MSH2 was demonstrated by co-immunoprecipitation experiments in which complex formation was found to be mispair dependent; presumably the complexes formed contained MSH3 or MSH6 although this was not demonstrated directly [47•]. These interactions required ATP although it is not clear whether the crucial interaction with ATP involved the ATP-binding site of the MutL homologues or the MutS homologues or both.

S. cerevisiae contains genes encoding two additional MutL homologues, MLH2 and MLH3 [8,48]. MLH2 does not appear to be involved in MMR [48]. A fragment of MLH3

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was found to interact with MLH1 and this interaction appeared to require the region of MLH1 known to interact with PMS1 [45]. Experiments with the full-length proteins demonstrated that MLH1 and MLH3 do indeed form a complex like MLH1 and PMS1. Mutations in *MLH3* caused a weak mutator phenotype by themselves and caused a striking increase in the rate of accumulation of frameshift mutations when the *MSH6* gene was also inactivated. The MMR defect seen in *mlh3 msh6* double mutants was not as strong as seen in *msh2* mutants. These data suggest that the MLH1–MLH3 complex plays a role in the repair of insertion/deletion mispairs by the MSH2–MSH3 pathway although only a portion of such repair requires MLH1–MLH3. Interestingly, the requirement of MLH1–MLH3 varied considerably depending on the frameshift site analyzed [48].

What roles do *E. coli* MutL and its eukaryotic homologues play in MMR? Besides interaction with MutS bound to a mispair, *E. coli* MutL is able to bind and hydrolyze ATP, and the homology relationships between MutL and the eukaryotic MutL homologues suggest that this is a conserved property [49•]. No other activity has been demonstrated for MutL but it has been demonstrated to activate the endonuclease activity of MutH and the helicase activity of UvrD (MutU, helicase II) [50–52]. Although litte data is available in this regard for the eukaryotic MutL homologues, the observed interactions between the MutL homologue complexes and the MSH complexes is consistent with this possiblilty. An additional role of MutL and the MutL homologues may be in enhancing mismatch recognition. MutL increases the efficiency of MutS recognition of a mismatch [53] and similar results have been obtained with *S. cerevisiae* MLH1–PMS1 [46]. The observation that MLH1–MLH3 only functions in the repair of some mispairs but not others could be explained if the MLH complexes play some role in mismatch recognition. Alternatively, the MLH complexes could recognize some conformational feature of MSH complexes that is determined in part by the exact mispaired base being recognized.

Other proteins implicated in mismatch repair

One of the important issues in MMR is the identification of the other proteins required for this process. Considerable progress has been made in this area recently, particularly with regard to the identification of exonucleases (Exonuclease 1, FEN1 (RAD27), and DNA polymerases δ and ε) and DNA replication factors (DNA polymerase δ, RPA [replication protein A], PCNA [proliferating cell nuclear antigen] and RFC [replication factor C]) that might function in MMR.

Exonuclease 1 was originally described in *S. pombe* and was suggested to play a role in MMR because of the effects that mutations in the *EXO1* gene had on recombination of closely linked markers [54]. Additional evidence that EXO1 might function in MMR came from the identification of the *S. cerevisiae EXO1* gene, which encodes a protein that interacted with MSH2 [55]. Mutations in *S. cerevisiae EXO1* caused weak mutator phenotypes consistent with the view that Exonuclease 1 might be one of a number of partially redundant exonucleases that function in MMR as suggested from studies in *E. coli*. Additionally, epistasis analysis supported the view that Exonuclease 1 might function in the same pathway as MSH2. Exonuclease 1 is a 5′→3′ exonuclease that has a preference for degrading double-stranded DNA [56]. One implication of this is that a DNA helicase might not be required for the 5′→3′ degradation step in eukaryotic MMR contrary to the case in *E. coli*. The human and mouse *EXO1* genes have been described recently ([57–59]; D Tishkoff, W Edelmann, R Kucherlapati, R Kolodner, unpublished data). The human gene encodes two forms of the protein that differ at the carboxyl terminus as a result of alternative mRNA splicing [57] and, like *S. cerevisiae* Exonuclease 1, the human protein also interacts with MSH2 [59].

A second exonuclease that has been implicated in MMR is the endo/exonuclease FEN1 (RAD27) that was originally purified as an activity that cleaves branched DNA structures [60]. This protein has been shown to play an important role in processing the 5′ ends of Okazaki fragments [61,62] and in the repair of alkylation damage to DNA [63,64]. Mutations in *RAD27* were shown to cause a strong mutator phenotype that was originally thought to be caused by a defect in MMR [65]. Subsequent analysis showed that this mutator phenotype was almost entirely caused by the accumulation of duplication mutations, which were postulated to be caused by defects in Okazaki fragment processing [66•]. It was also observed that *rad27* mutations caused a small increase in the rate of occurrence of frameshift mutations like those that arise in MMR-defective mutants [66•]. The increase in the rate of frameshift mutations was ~5% of that seen in an *msh2* mutant and on the order of that seen in *exo1* mutants [9,55]. This raises the possibility that FEN1 (RAD27) may also be a partially redundant endo/exonuclease that acts in MMR. It is worth noting that FEN1 (RAD27) interacts with PCNA, a protein that interacts with other MMR proteins [44].

A number of DNA replication factors have been implicated in MMR. Studies in which extracts of human cells were fractionated to identify factors required for MMR have pinpointed DNA polymerase δ as being required for DNA synthesis during MMR [67]. Similarly, immunodepletion and reconstitution experiments have demonstrated that the single-stranded DNA-binding protein RPA is required for MMR [68]. A number of experiments have implicated the replication factor PCNA in MMR. These include studies indicating that PCNA interacts with several different MMR proteins [47•], the description of PCNA mutants having mutator phenotypes [69,70], and biochemical studies indicating that PCNA is required at both early stages of MMR [47•,70] and during MMR-associated DNA synthesis [47•]. Although both the genetic and protein interaction studies published to date seem incomplete, the sum of all

of the available data provides a compelling argument that PCNA plays a central role in MMR. Given the involvement of PCNA, it might be reasonably presumed that RFC, the factor required to load PCNA onto DNA, is also required for MMR [71].

The progress to date suggests that, as in *E. coli*, many of the proteins required for eukaryotic DNA replication are also required for the DNA synthesis steps of MMR. A number of unanswered questions remain as to which other proteins are required for MMR. The genetics of *EXO1* and *FEN1* (*RAD27*) are consistent with the involvement of multiple partially redundant exonucleases in MMR. It remains an open question whether there are other 5′→3′ exonucleases that act in MMR. Furthermore, 3′→5′ exonucleases that might act in MMR have not yet been identified, although it has been suggested that the 3′→5′ editing exonuclease functions of DNA polymerases δ and ε might provide this function [72]. An intriguing question raised by the likely requirement of multiple exonucleases, all of which act on double-stranded DNA templates, is whether eukaryotic MMR requires a DNA helicase. It is possible that eukaryotic MMR may differ from prokaryotic MMR, which requires the combined action of single-stranded DNA exonucleases and a DNA helicase [2,3].

An important question that remains unanswered is how eukaryotes distinguish between parental and daughter DNA strands during MMR as MutH homologues appear to be absent in eukaryotes. In this regard, results implicating PCNA in MMR are intriguing. The fact that PCNA interacts with both MMR and replication proteins, and that PCNA is required at both early and late stages of MMR suggests that PCNA participates in the initiation of MMR. Several possibilities are consistent with the available results. PCNA may be involved in assembling a complex of proteins at the mispair, or elsewhere on the DNA which is required for the initiation of MMR [47•,70]. Proteins involved in strand discrimination and possibly mismatch-dependent incision could be part of such a complex. Second, PCNA could couple MMR proteins to replication proteins at the replication fork. As PCNA is asymmetric and is loaded onto the DNA with a polarity during DNA replication, PCNA could even couple MMR proteins to the daughter strand directly and thus itself be an important factor in strand discrimination. It is also possible that the involvement of PCNA in MMR is simply as a result of its role as a cofactor for DNA polymerase δ. Clearly, much work remains to be done in this area

Other functions for mismatch repair proteins

A number of studies have indicated that MMR proteins function in processes other than MMR — one being the repair of branched DNA structures. MSH2 and MSH3 are known to be required along with the RAD1–RAD10 endonuclease for the processing of non-homologous ends during certain types of recombination [73•]. Similarly, repair of a 26 base insertion/deletion mispair formed during meiotic recombination required both MSH2 and RAD1–RAD10 [18]. It has been shown that MSH2 and MSH2–MSH6 can bind to Y-structures, Holliday junctions and a variety of types of DNA damage normally thought to be repaired by other repair pathways such as nucleotide excision repair (NER) [74–76] (GT Marsischky *et al.*, unpublished data). MSH2 and presumably the MSH complexes have been shown to interact physically with RAD1–RAD10 and other NER proteins [77]. These results suggest that the MSH complexes could function in some types of repair and recombination reactions distinct from MMR by targeting proteins other than MMR proteins such as RAD1–RAD10 to different types of DNA structures during different types of DNA repair.

It has been known from studies in bacteria for some time now that MMR can prevent recombination between divergent sequences. The picture has been more opaque in eukaryotes and in particular *S. cerevisiae* because defects in MMR were initially shown to only cause small increases in recombination between divergent sequences. More recently, it has been shown that there are two stages at which regulation of recombination between divergent sequences occurs [78,79]; first, at higher levels of sequence divergence, the homologous pairing is limited; and second, at low levels of sequence divergence where homologous pairing can occur efficiently, MMR acts to greatly reduce the formation of mature recombinants. Exactly how this occurs is less clear but the ability of MMR proteins to interact with branched DNA structures like Holliday junctions as well as with recombination proteins is providing insight into this.

Implications for cancer genetics

HNPCC is caused by inherited mutations in MMR genes. Mutations in HNPCC families found to date are in the *MSH2* and *MLH1* genes with mutations in other genes such as *MSH3*, *MSH6*, *PMS2* (*S. cerevisiae PMS1*) and *PMS1* (*S. cerevisiae MLH3*) being either rare or non-existent [1]. The observed redundancy between *MSH3* and *MSH6* has provided an explanation for why mutations in these genes were not initially found in HNPCC families [4,9]. More recently, the observation that mutations in *MSH6* cause only very low rates of accumulation of frameshift mutations in repeated sequences like mono- and dinucleotide repeat sequences but nonetheless cause significant cancer susceptibility in mice [14•] has provided a basis for the initial identification of germline *msh6* mutations that are associated with cancer susceptibility [80,81]. How prevalent such mutations will prove to be is unclear at present. An outstanding question is the low frequency of germline mutations in *PMS1* and *PMS2* found in HNPCC families. It is possible that the above discussed partial redundancy between *S. cerevisiae PMS1* (human *PMS2*) and *MLH3* (human *PMS1*) may help explain the low frequency of human *PMS1* and *PMS2* mutations [48] but this partial redundancy is subtle. The suggestion is that a crucial target gene must exist, the

inactivation of which is necessary for cancer development, that contains a mutable sequence in which frameshift mispairs would be relatively equally reparable by PMS1- and PMS2-dependent MMR. In this regard, it is worth noting that mice containing mutations in *PMS1* (*S. cerevisiae MLH3*) have only weak mutator phenotypes [82].

Another issue in the study and diagnosis of HNPCC is the interpretation of missense mutations. Analysis of the recently published crystal structure of a fragment of MutL [49•] has shown that many but not all *mlh1* missense mutations found in HNPCC families may map to conserved residues thought to be important for the ATPase activity of MutL [49•]. Future studies of this type along with functional studies [83] will be helpful in continuing to evaluate the significance of such missense mutations.

Conclusions

Considerable progress has been made in identifying and understanding the proteins that function in eukaryotic MMR but this proces has not yet been reconstituted with purified proteins nor have all of the proteins required for it been identified. Although several novel models relating to the mechanism of MMR have been proposed, there is only a limited understanding of its mechanism in eukaryotes and in particular how it is targeted to the daughter strand. Evidence has accumulated demonstrating that MMR proteins can function in other repair processes although the mechanisms of these reactions are also not well understood. Given the relationship between MMR defects and cancer susceptibility and the current excitement in the field, progress in answering these questions is expected to be rapid.

Note added in proof

The work referred to as GT Marsischky *et al.*, unpublished data, is now in press [84].

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